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Transketolase from *Escherichia coli*: A Practical Procedure for Using the Biocatalyst for Asymmetric Carbon-Carbon Bond Synthesis

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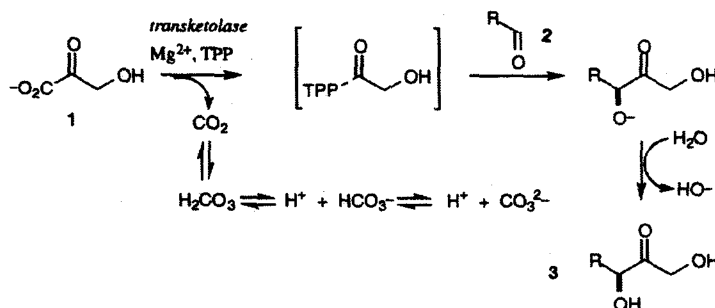
Abstract: A practical procedure is reported for the use of the enzyme transketolase, from *Escherichia coli*, for asymmetric carbon-carbon bond synthesis. The reactions with the biocatalyst are conveniently carried out, on a gram scale, in unbuffered aqueous media by employing a pH autotitrator. An improved large scale synthesis of hydroxypyruvate is also reported. Copyright © 1996 Elsevier Science Ltd

Enzymes have proven to be useful catalysts for asymmetric carbon-carbon bond synthesis.[1] Although most effort has been directed towards the application of aldolases[2] for the stereospecific addition of a 3-carbon unit, equally important is enzymic 2-carbon (transketolase[3a-d]) and 1-carbon (oxynitrilase[4]) chain extension. Despite the obvious attractions of enzyme-mediated C-C bond synthesis, a number of problems need to be solved, in particular the requirement for phosphorylated substrates (aldolases), the use of toxic cyanide (oxynitrilase), the availability of large quantities of the enzymes involved, and the recovery of the products from aqueous buffered media. With these considerations in mind we have selected the enzyme transketolase (TK) for development as a practical biocatalyst for enzyme-catalysed asymmetric carbon-carbon bond synthesis on a large scale.[5] Herein we report a convenient method for carrying out these reactions in unbuffered solutions together with protocols for the synthesis of one of the required substrates on a practical scale.



Scheme 1: Transketolase catalysed reaction.

Transketolase catalyses the reaction of hydroxypyruvate **1** with an aldehyde **2** to yield a keto-diol **3** (**Scheme 1**). The TK gene has been over-expressed in *E. coli* resulting in a transformant that produces TK as ≈40% of the soluble protein.[6] Preparation of a cell-free system followed by removal of cell-debris and one-step ammonium sulphate fractionation yields an extract that is ~ 70% pure and suitable as a catalyst for the biotransformations. The reaction (**Scheme 1**) is rendered irreversible by the release of CO_2 from hydroxypyruvic acid **2** and is accompanied by an increase in pH due to the consumption of one proton per molecule of product formed, presenting a convenient method for monitoring the progress of the reaction.[7]

We have previously shown that *E.coli* TK catalyses the condensation of a range of aldehydes[3a,8], particularly those containing an α-hydroxyl group in the D (*i.e.* 2*R*)-configuration. The high stereoselectivity for the *R*-enantiomer was demonstrated by the individual biotransformations with D-, D/L-, and L-glyceraldehyde (**Figure**). It can be seen that with L-glyceraldehyde there is essentially no reaction but that D-glyceraldehyde reacts at approximately the same rate as the achiral substrate glycolaldehyde.[9]

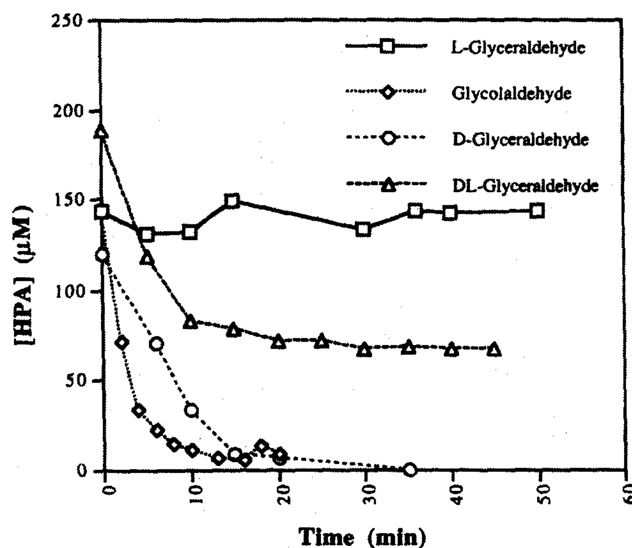


Figure: Enantioselectivity of *E. coli* transketolase towards D-, L- and DL-glyceraldehyde.

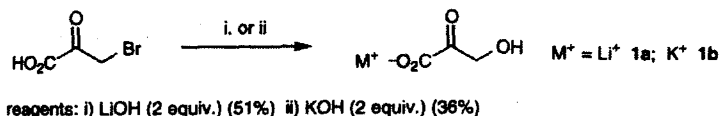
Previously we[3a] and others[3c,3d] have reported carrying out these TK catalysed reactions in buffered media (100 mM, pH 7.0) using either glycylglycine or Tris.HCl. In our experience, this led to problems of reaction of the aldehyde with the buffer, presumably *via* Schiff's base formation, and hence the requirement for addition of excess aldehyde. Whitesides *et al.*,[3b] have reported a method that involved addition, on demand, of hydroxypyruvic acid to control the pH as the reaction progressed. Although we have found this method to be reproducible, the free acid must be prepared from the salt by ion-exchange prior to use, and hence we endeavoured to devise a simpler protocol. We have found that the reaction can be performed in an unbuffered medium, using lithium or potassium hydroxypyruvate directly, and automatically maintaining the pH at 7.0 by the controlled addition of 1 M HCl through the use of an autotitrator. This protocol not

only resulted in improved yields (**Table**) and ease of work-up but also provided a convenient method for determining the end-point of the reaction. The increase in pH during the reaction is partially offset by the dissolution of carbon dioxide producing carbonic acid. Thus the reaction comes to completion before the addition of the theoretical amount of 1 M HCl (*e.g.* for glycolaldehyde **2c** the end point was reached after addition of 85% of the theoretical amount of 1 M HCl). In order to evaluate this simplified method, we selected for optimisation the reaction of 2*RS*, 3-*O*-benzylglyceraldehyde **2a**[10] to give 5-*O*-benzyl xylulose **3a**.[11] The aldehyde substrate can be prepared in *situ*, by acid catalysed hydrolysis of the corresponding diethylacetal, and used directly in the biotransformation. Its low solubility in water is typical of many of the substrates used with TK but significantly we have found that the addition of THF (<10% v/v) as a co-solvent to aid solubility has little effect on the stability of the enzyme. The reaction can be carried out at concentrations up to 0.14M on a gram scale resulting in an isolated yield of 76% of the product **3a**.

Table: Results of *E. coli* transketolase catalysed couplings in unbuffered medium (yields in brackets are for corresponding reactions in 0.1M glycylglycine buffer, pH 7.0).

aldehyde	product(s)	yield/%
2a	**3a**	76
2b	**3b**	23 (12)
2c	**3c**	74 (43)
2d	**3d**	33:18 (26)
2e	**3e**	29 (23)

Although the use of hydroxypyruvate results in irreversible conversion to products, and hence is preferred to other alternative ketoses as the C-2 ketol donor, its high cost and difficulty of preparation currently poses a limitation on the scale-up of the reaction. The reported synthesis of lithium hydroxypyruvate from bromopyruvic acid[12] suffers from poor overall yield (23% on a 2.5 g scale) and low concentration of the product (2.5 g from 235 ml of water). In our hands, slight modification of this procedure by elimination of the final recrystallisation step, yielded pure (>95%) lithium hydroxypyruvate on a 25 g scale in 51% yield (**Scheme 2**). However, attempts to increase the concentration of product were unsuccessful resulting in precipitation of the intermediate lithium bromopyruvate. This problem was overcome by switching to the potassium salt which was isolated in 36% yield at higher product concentration [39 g (0.24 mol) per litre of reaction volume versus 18 g (0.14 mol) per litre for lithium hydroxypyruvate]. The potassium salt behaves identically to the lithium salt in the biotransformation reactions and hence we recommend its use.[13]



Scheme 2: Synthesis of hydroxypyruvate

Acknowledgement: We are grateful to the BBSRC for the provision of research grants (NJT JMW).

References and Notes:

1. *Enzymes in Organic Synthesis*, Ed. G.M. Whitesides and C.-H. Wong, Pergamon, 1994.
2. W.D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G.E. Schulz, J. Badia, and J. Aguilar, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 555; I. Henderson, C.-H. Wong, and K.B. Sharpless, *J. Am. Chem. Soc.*, 1994, **116**, 558.
3. Transketolase can be obtained from a number of different sources; (*E. coli*) [a]G. R. Hobbs, M. D. Lilly, N. J. Turner, J. M. Ward, A. J. Willetts and J. M. Woodley, *J. Chem. Soc. Perkin Trans. I*, 1993, 165; (yeast) [b]Y. Kobori, D. C. Myles and G. M. Whitesides, *J. Org. Chem.* 1992, **57**, 5899; (yeast) [c]F. Effenberger, V. Null and T. Ziegler, *Tetrahedron Lett.* 1992, **33**, 5157; (spinach) [d]J. Bolte, C. Demuynck, L. Hecquet and V. Dalmas, *Tetrahedron Lett.* 1991, **32**, 5085.
4. F. Effenberger, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 458; N. Klempier, H. Griengl, and M. Hayn, *Tetrahedron Lett.*, 1993, **34**, 4769; V.I. Ognyanov, V.K. Datcheva, and K.S. Kyler, *J. Am. Chem. Soc.*, 1991, **113**, 6992.
5. G. R. Hobbs, M. D. Lilly, N. J. Turner, J. M. Ward and J. M. Woodley, *Proc., 6th Eur. Cong. Biotechnol.*, 1994, 463.
6. C. French and J. M. Ward, *Biotechnology Letters*, 1995, **17**, 3, 247.
7. G. Hübner and R. Golbik, *Biochemistry International*, 1992, **26**, 545.
8. A.J. Humphrey, N.J. Turner, R. McCague, and S.J.C. Taylor, *J. Chem. Soc., Chem. Commun.*, 1995, 2475.
9. HPA assayed by the reported method; A.W. Holldorf, in *Methods in Enzymology*, Academic Press, New York, 1966, Volume 3, p.578.
10. *Example procedure: Biotransformation of 3-O-benzylglyceraldehyde **2a** in unbuffered medium;* 2*RS*, 3-*O*-Benzylglyceraldehyde diethyl acetal (3.5 g, 13.8 mmol) was suspended in aq. HCl (0.1M, 100 cm^3) and stirred at room temp., for 30 h, after which TLC indicated complete conversion to product yielding a turbid solution of 2*RS*, 3-*O*-benzylglyceraldehyde **2a**. The reaction mixture was then diluted by addition of water (200 cm^3) after which the pH of the solution was adjusted to pH 7.0, by careful titration with aq. NaOH (5M), followed by the addition of thiamine pyrophosphate (150 mg, 0.326 mmol), and magnesium chloride (13 mg, 78 mmol). Readjustment of the pH to 7.0 by addition of aq. NaOH (0.1M) was followed by the addition of transketolase (600 U, 3 cm^3) and bovine serum albumin (50 mg). The reaction was initiated by the addition of potassium hydroxypyruvate (0.98 g, 6.9 mmol) and the pH maintained at 7.0 by auto-titration with aq. HCl (1M). After 20 h, THF (20 cm^3) was added to aid solubility of the remaining aldehyde and hence ensure complete reaction. The reaction was terminated after 24h by which time uptake of aq. HCl had ceased. Addition of flash silica (~4g) followed by removal of the water/THF under reduced pressure and flash chromatography (ethyl acetate-petrol = 2:3 then ethyl acetate-methanol = 9:1) afforded 5-*O*-benzyl-D-xylulose **3a** (1.262 g, 5.3 mmol, 76%).
11. We are currently utilising the triol **3a** for the synthesis of target natural products, details of which will be published in due course.
12. F. Dickens, *Biochemical Preparations*, 1962, **9**, 86.
13. *Large scale synthesis of potassium hydroxypyruvate*; Bromopyruvic acid (80 g, 0.48 mol), freshly recrystallised from chloroform, was dissolved in H_2O (400 cm^3) followed by careful addition of aq. KOH (5M) until a stable pH of 9.0 was achieved. At no time during the addition was the pH allowed to exceed 9.5. The reaction mixture was then adjusted to pH 5.0 by addition of glacial acetic acid and concentrated under vacuum until crystals of the product had just begun to appear. Subsequent refrigeration overnight, filtration and drying over phosphorus pentoxide afforded highly crystalline potassium hydroxypyruvate (24.46g, 0.17 mol, 36%).

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